カビ処理したサバ廃棄油を与えたワムシの脂肪酸組成および餌料価値

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Fatty Acid Composition and Dietary Value of Rotifer *Brachionus plicatilis* Fed on Waste Lipid Treated with *Aspergillus terreus*[*1]

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Attempts were made to improve the dietary value of rotifer fed on baker's yeast by direct feeding of microbial-treated lipid. The nontreated lipid (NTL) from mackerel waste juice was treated with *Aspergillus terreus*, and the treated lipid (TL), NTL and pollack liver oil (PLO) were emulsified with sea water and proteinous sediment from the waste juice. The emulsified lipids and yeast were fed to rotifers and the proximate and fatty acid compositions of the rotifers were determined. Furthermore, ayu larvae were reared with the rotifers fed on baker's yeast (BY) with or without TL or NTL, and their growth and survival rate were compared. The rotifers fed on the lipids showed similarly higher lipid content than the initial rotifer. However, the ω3 HUFA content of rotifer fed on TL (BY+TL rotifer) was higher than that of the rotifer fed on NTL (BY+NTL rotifer) and similar to rotifer fed on PLO (BY+PLO rotifer). The total body length of larvae reared with BY+NTL rotifer (BY+NTL group) was significantly inferior to that of BY+TL group at the end of the 25 days feeding trial.

The survival rates of BY+NTL group in feeding trial and handling tests were also lower than those of BY+TL group. The larvae reared with rotifer fed on BY only were the lowest of all the groups in the growth and survival rate.

Juice from pressed mackerel waste consists of liquid, lipid and proteinous sediment. These substances become a source of pollution. Therefore, it is desirable to utilize them without discarding.

The studies showed that the waste lipid could be used as a feed oil by the treatment with *Aspergillus terreus*[1] and the liquid as a nutrient source for yeast.[2] However, the ω3 highly unsaturated fatty acids (ω3 HUFA) content of rotifer fed on yeast cultured in sea water containing the liquid from mackerel waste juice or commercial baker's yeast was lower than that of rotifer fed on ω-yeast (Kyowa Hakko Co. LTD.) or marine chlorella.[3]

Other investigation[4] pointed out that ω3 HUFA content in live feeds is principal factor in their dietary value. It was also reported that the dietary value of rotifer reared with baker's yeast was improved by direct feeding of pollack liver oil emulsified with sea water and raw egg yolk etc.[5] However, commercial feed oil and synthetic emulsifier are relatively costly.

On the other hand, the microbial treatment with *Asp. terreus* decreased the peroxidized products in waste lipid and increased the ω3 HUFA content and improved the nutritive value of the lipid.[1] Furthermore, it is considered that the proteinous sediment in waste juice can be used as an emulsifier.

In this study, therefore, in order to improve the dietary value of rotifer reared with baker's yeast, the microbial-treated waste lipid, which was emulsified with the proteinous sediment and sea water, was fed directly to rotifer, and the dietary value of rotifer was estimated on the bases of the ω3 HUFA content and effect of rotifer on the growth, survival rate and activity of ayu larvae.

Materials and Methods

Treated and Nontreated Lipids

Fifty grams of lipid (nontreated lipid) from pressed mackerel waste juice was treated with 50 m*l* of *Asp. terreus* seed by the procedure described in the previous paper,[1] and the treated lipid was separated by centrifuging.

The peroxide values (POV), thiobarbituric acid (TBA) values and fatty acid compositions of nontreated and treated lipids are shown in Table 1. The POV and TBA value of treated lipid were

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Table 1. POV, TBA values, and fatty acid compositions of nontreated (NTL) and treated (TL) waste lipids, and pollack liver oil (PLO)

Fatty acid	NTL	TL	PLO
12: 0	1.1	0.4	0.1
13: 0	0.8	0.2	tr
14: 0	8.4	5.8	5.3
15: 0	1.0	0.8	0.3
16: 0	22.5	19.0	8.6
16: 1ω7	5.1	5.3	7.7
18: 0	4.9	3.7	2.4
18: 1ω9	21.2	20.4	13.5
18: 2ω9	0.3	0.7	1.0
18: 3ω6	tr	tr	0.4
18: 3ω3	0.8	1.0	tr
18: 4ω3	—	—	0.5
20: 1ω9	7.7	8.8	18.2
20: 1ω7	1.0	1.7	2.9
20: 2ω9	1.3	1.1	0.2
20: 2ω6	—	—	0.2
20: 3ω3	—	—	0.1
20: 4ω6	0.8	0.8	0.3
20: 5ω3	3.2	5.1	10.7
22: 1	6.9	7.7	14.2
22: 4ω6	1.0	0.5	0.6
22: 5ω6	1.3	2.0	1.0
22: 5ω3	0.6	1.4	0.9
22: 6ω3	5.0	6.6	7.3
$\Sigma\omega$3 HUFA	8.8	13.1	19.0
POV (meq/kg)	228.5	58.6	
TBA (mg/kg)	78.9	38.7	

considerably lower than those of nontreated lipid, and the percentage of ω3 HUFA in total fatty acids was remarkably higher.

Emulsions of Lipids

A preliminary experiment was conducted to examine whether the sediment from pressed juice acts as an emulsifier on the mixture of sea water and nontreated or treated lipids, and the stability of this emulsion was compared with that of the emulsion of treated lipid and sea water added with raw egg yolk. Consequently, it was found that a relatively stable emulsion was obtained when 5 g of nontreated or treated lipid, 90 m*l* of sea water and 5 g of sediment were blended with a homogenizer at 10,000–15,000 rpm for 10–15 min. Therefore, two emulsions of nontreated and treated lipids were prepared on this condition.

Rotifer

Rotifer was reared with baker's yeast during the prefeeding period by the procedure mentioned previously.[3] When the density of rotifer reached approximately 280–300 indv. per m*l*, the rotifer was filtered through a plankton net with 60 μ mesh. The filtered rotifer was released into polyvinyl tanks of 100 *l* capacity with 80 *l* of filtered sea water, at a density of about 1,000 indiv./m*l* per tank. Each tank was sufficiently aerated. The rotifer in each tank was fed on 20 g of backer's yeast alone (BY) or the mixtures of 20 g of baker's yeast and 100 m*l* of emulsified-nontreated lipid (BY+NTL) or emulsified-treated lipid (BY+TL), respectively, for 12 h.

Ayu Larvae

Ayu eggs were kept in 1 t transparent polyvinyl tanks. The tanks were supplied with water sterilized by ultraviolet ray at a rate of 3 *l* per min and aerated at a rate of 600 m*l* per min, respectively. Moreover, 1 ppm malachite green was added daily into the water of each tank until hatching. A photo-period of 12L-12D was maintained with ordinary electric lamps and a regulator. The hatching began on 15th day after fertilization and continued for 24 h.

The larvae were bathed in 1 ppm sodium nifurstyrenate, and the water was switched to sea water sterilized by ultraviolet ray after the hatching completed. The mouth of larvae opened on 3rd day after hatching. Then, rotifers reared with yeast were fed to the larvae in each tank at a rate of 10 indiv. per m*l* until grouping.

Grouping and Rearing of Fish

On 8th day after hatching, the larvae averaging 8.0 mm in total length were divided into 3 groups with each 1,000 larvae and were kept in 100 *l* black polyvinyl tanks. On the other hand, the rotifers reared with BY, BY+NTL and BY+TL were bathed in 1 ppm sodium nifurstyrenate for 1 h after washing with sterilized sea water and were fed to the larvae in each tank at a rate of 10 indiv. per m*l* daily for 25 days. A tank was continuously supplied with sea water sterilized by ultraviolet ray at a rate of 600 m*l* per min and aerated (50 m*l* per min). A 12L-12D photo-period was maintained with ordinary electric lamps and a regulator. The water surface had 2,000 lux in the light period. Water temperature declined gradually from 17.5°C at the biginning of feeding trial to 12.5°C at the end. The feces, dead larvae and rotifer were slowly siphoned out twice a day and the dead larvae were counted.

Twenty five larvae were sampled at random from

each tank every 5 days except that 50 larvae were sampled at the end of feeding trial, and their total body lengths were measured with a shadow meter.

Handling Test

After the feeding trial, in order to estimate the activity of ayu larvae, the larvae were subjected to two handling tests. In the test I, all alive larvae of each group were transferred carefully to other tanks with a scoop of water and kept for 24 h without feeding. Water supply and aeration were maintained as in the feeding trial. During the keeping period, the dead larvae were siphoned out and counted.

After the first handling test, the larvae of each group were fed on the corresponding rotifer for 2 days. The alive larvae were dipt out of water with a net for 5–6 s after the feeding period and then were again kept in water for 24 h without feeding. The dead and alive larvae were counted (test II).

Table 2. Proximate compositions of rotifers (BY+NTL, BY+TL and BY+PLO) fed on baker's yeast (BY) and nontreated waste lipid (NTL), treated waste lipid (TL), or pollack liver oil (PLO)

Rotifers / Feeding time	Initial (before feeding)	BY+NTL		BY+TL		BY+PLO	
		6 h	12 h	6 h	12 h	6 h	12 h
Moisture (%)	89.1	87.2	86.8	87.8	86.9	87.3	86.9
Lipid* (%)	2.0	2.9	3.2	2.8	3.3	3.0	3.2
	(18.3)	(22.7)	(24.2)	(23.0)	(25.2)	(23.6)	(24.4)
Protein* (%)	6.8	7.4	7.5	7.3	7.2	7.3	7.2
	(62.4)	(57.8)	(54.3)	(59.8)	(55.0)	(57.5)	(55.0)
Ash* (%)	0.5	0.5	0.6	0.4	0.5	0.5	0.5
	(4.5)	(3.9)	(4.3)	(3.3)	(3.8)	(3.9)	(3.8)

* Values in parenthesis indicate % on dry matter basis.

Table 3. Changes in fatty acid compositions of lipids in rotifers (BY+NTL, BY+TL and BY+PLO) fed on baker's yeast (BY) and nontreated waste lipid (NTL), treated waste lipid (TL). or pollack liver oil (PLO)

Rotifers / Feeding time	Initial (before feeding)	BY+NTL		BY+TL		BY+PLO	
		6 h	12 h	6 h	12 h	6 h	12 h
12:0	0.7	0.3	0.5	0.4	0.4	0.3	0.2
13:0	0.5	0.4	0.6	0.7	0.4	0.3	0.2
14:0	1.6	2.7	2.7	3.3	3.2	2.5	3.8
15:0	0.7	0.9	0.7	1.1	1.0	0.9	0.8
16:0	6.3	10.6	7.1	8.0	8.2	8.2	9.0
16:1ω7	18.3	16.0	16.6	13.5	15.1	15.2	13.3
18:0	5.4	5.5	5.5	5.6	5.1	4.8	4.0
18:1ω9	31.0	31.4	34.2	28.7	30.7	28.6	27.2
18:2ω6	8.9	6.1	5.6	5.1	6.1	6.2	7.2
18:2ω4	0.8	1.1	1.1	1.2	0.9	0.9	0.8
18:3ω3	1.2	1.9	1.4	1.5	1.8	2.1	1.7
20:1	4.5	4.3	4.7	5.1	5.7	8.6	9.0
20:2ω9	—	0.8	1.0	1.2	0.7	0.9	1.1
20:2ω6	2.6	0.9	1.1	1.8	0.7	1.0	0.8
20:3ω6	0.5	0.7	1.2	1.0	0.5	0.2	0.3
20:4ω3	1.1	0.9	1.3	1.2	1.2	0.6	0.7
20:5ω3	1.1	1.5	1.4	2.4	2.5	2.9	4.3
22:1	2.5	3.2	3.0	4.3	3.9	4.7	5.6
20:4ω6	1.1	0.7	1.0	1.0	1.2	1.0	1.1
22:5ω6	2.3	1.2	1.5	1.6	2.1	1.5	0.9
22:5ω3	—	0.5	0.7	0.9	1.8	1.1	0.7
22:6ω3	—	1.0	1.2	2.6	2.4	2.2	2.3
$\Sigma\omega$3 HUFA	2.2	3.9	4.6	7.1	7.9	6.8	8.0

Table 4. Effects of rotifers fed on baker's yeast (BY), nontreated waste lipid and BY (BY+NTL), or treated waste lipid and BY (BY+TL) on growth and survival rate of ayu larvae

		Rotifers fed on		
		BY	BY+NTL	BY+TL
No. of larvae	at start	1000	1000	1000
	after 25 days	(100)*1	(100)	(100)
Survival rate (%)		326	418	475
		36.2	46.4	52.8
Total body length (mm)	at start	8.0±0.6	8.0±0.6	8.0±0.6
	after 10 days	9.9±0.7	11.2±1.0	11.7±0.9
	after 25 days	12.6±1.5	13.6±1.5	15.9±2.1
"t" test*2	(5%)	IS	—	S

*1 number in parentheses indicated the larvae sampled.
*2 S: significant, IS: insignificant.

Analytical Methods

The proximate compositions of rotifers before and after 6 or 12 h feeding were determined by the methods mentioned previously.[6] The fatty acid compositions of nontreated and treated lipids, pollack liver oil (Riken Vitamin Co. LTD.) and lipids of rotifers before and after feeding were analyzed by the methods described in the previous paper[7] except for the column temperature of 175°C in GLC operating conditions.

Results and Discussion

Changes in Proximate Compositions of Rotifers

As shown in Table 2, the rotifers fed on emulsified lipids (BY+NTL, BY+TL and BY+PLO rotifers) showed similarly high lipid and low protein contents as compared with the initial rotifer. Furthermore, the lipid content increased with the elongation of feeding period in every group.

Fatty Acid Compsitions of Rotifers

The percentages of ω3 HUFA in total fatty acids of rotifers reflected those of emulsified lipids fed (Tables 1 and 3). Namely, the percentage of ω3 HUFA in rotifer fed on the treated lipid (BY+TL rotifer) was higher than that of the nontreated lipid group (BY+NTL) and similar to the pollack liver oil group (BY+PLO). On the other hand, the rotifers (BY+NTL, BY+TL and BY+PLO) showed similar lipid contents at each feeding period (Table 2). Accordingly, it can be considered that the ω3 HUFA contents of rotifers also increased with the elongation of feeding period and reflected that of lipids fed. Watanabe *et al.*[5] also reported that the ω3 HUFA content in rotifer reached a maximum after 6–12 h feeding of emulsified lipid and was proportional to the content of ω3 HUFA in lipids emulsified with sea water and raw egg yolk, soybean lecithin or casein-Na. On the bases of these findings, rotifers fed on lipids for 12 h were employed as a feed for ayu larvae in the feeding trial.

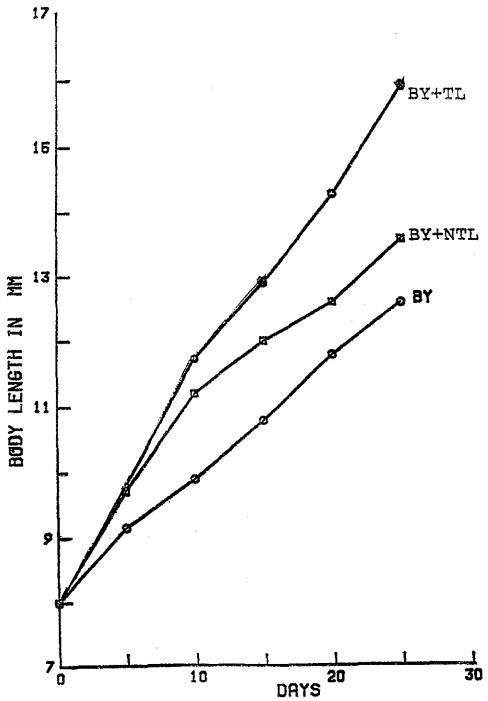

Fig. 1. Growth of ayu larvae fed on rotifers reared with baker's yeast (BY), nontreated waste lipid and BY (BY+NTL) or treated waste lipid and BY (BY+TL).

Growth and Survival Rate

The growth and survival rate of ayu larvae fed on BY rotifer reared with baker's yeast alone were the lowest of all the groups, as shown in Table 4 and Fig. 1. The growth of ayu larvae

Table 5. Effects of rotifers fed on baker's yeast (BY). nontreated waste lipid and BY (BY+NTL) or treated waste lipid and BY (BY+TL) on survival rate of ayu larvae in handling tests

Rotifers	BY	BY+NTL	BY+TL
Test I*1 (%)	79.3	82.6	88.2
Test 2*2 (%)	63.1	78.1	92.0

*1 Larvae were transfered to other tanks with a scoop of water and kept for 24 h without feeding.

*2 Larvae were dipt out of water ith a net for 5–6 s and then weerkept in water for 24 h without feeding.

(BY+NTL group) fed on BY+NTL rotifer reared with yeast and nontreated lipid was similar to that of larvae (BY+TL group) fed on BY+TL rotifer reared with baker's yeast and treated lipid up to the initial 10 day of feeding, but the subsequent growth gradually declined. Consequently, the singificant difference in total body length at the end of feeding trial was recognized between BY+NTL group and BY+TL group. The survival rate of the BY+NTL group was also lower than that of BY+TL group. Furthermore, the deformed backbone was recognized in 5 larvae of the BY group fed on BY rotifer and 3 larvae of the BY+NTL group, respectively, per 100 larvae sampled from each group. On the other hand, the deformed backbone was not observed in the BT+TL group.

The results of handling tests are shown in Table 5. The survival rates of ayu larvae in the tests I and II were low in the groups fed on the BY and BY+NTL rotifers as compared with the BY+TL group fed on BY+TL rotifer. Namely, the larvae fed on rotifer enriched ω3 HUFA by direct feeding of microbial-treated waste lipid was sturdy.

From these findings, it is clear that the microbial treated lipid and proteinous sediment from mackerel waste juice contributed to the improvement of dietary value of rotifer for ayu larvae.

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